IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: ANDERSON, Norman Leigh	)	Confirmation No: 6420
	)	
Application Serial No.: 10/676, 005) Group Art Unit: 1655		
	)	
Filed: October 02, 2003	)	Examiner: Jana A. Hines
	)	Attorney Docket: 15503-001US

For: ***HIGH SENSITIVITY QUANTITATION OF PEPTIDES BY MASS SPECTROSCOPY***

Declaration under 37 C.F.R. § 1.132

I, Dr. Fred Regnier, declare and say:

1. I am the J. H. Law Distinguished Professor of Analytical Chemistry at Purdue University in West Lafayette, Indiana.

2. A copy of my *Curriculum Vitae* is appended below as APPENDIX A.

3. Much of my work over the last 25 years has focused on the separation and characterization of proteins. Presently, my laboratory is developing integrated analytical systems for the analysis and characterization of complex protein mixtures using multidimensional separation systems and mass spectrometry.

4. I am the senior author of a paper by Geng, Ji and Regnier[1] published in the Journal of Chromatography A, 870: 295–313 (2000)(the "Geng" reference), and also the inventor on related US Patents 6,872,575 and 6,864,099, and US Patent application 2002/0037532.

5. I am familiar with the methods described in the captioned application, which are known in the field as "SISCAPA" (= Stable Isotope Standards with Capture by Anti-Peptide Antibody). I have reviewed the claims set forth in Appendix B, and I have reviewed the portions of the Office Action mailed December 8, 2008 that refer to the Geng paper.

6. I first learned about the SISCAPA method and saw initial results described by Leigh Anderson at a meeting we both attended in Hinxton, near Cambridge UK, in the summer of 2005. After Dr. Anderson's lecture I mentioned to him that I wished I had thought of the method.

[1] Geng *et al.*, J. Chromatography A,. 870:295-313 (2000).

7. The SISCAPA approach using anti-peptide antibodies, some embodiments of which are set forth in the claims in Appendix B, was contrary to the general desire in the field at that time to detect large numbers of peptides, including both expected and potentially novel peptides. Instead, SISCAPA focused on a number of pre-selected signature peptides.

8. The Geng paper cited by the USPTO in the above-referenced application is the result of research that I directed in my laboratory at Purdue University. In it, we established the concept that tryptic peptides may be used as analytical surrogates for the protein from which they were derived, calling these "signature peptides".

9. Proteolytic digests of biological samples, such as blood serum or plasma, produce peptide mixtures of great complexity. To reduce this complexity, the methods described in the Geng paper used a fractionation process as part of the effort to detect the many peptides present. Specifically, Con A lectin affinity chromatography was used to enrich the class of glycopeptides. This method does not isolate specific peptides, but rather separates *classes* of peptides. Selected classes of peptides could then be further fractionated by reversed phase liquid chromatography (RPLC), followed by MALDI-TOF mass spectrometry to identify specific peptides in the RPLC fractions.

10. I recognized that other classes of peptides could be selected by affinity methods. For example, additional lectins other than concanavalin A could be used for glycopeptides, labeling of cysteines with an alkylating agent could be used to select cysteine containing peptides, or antibodies specific for a post-translational modification such as phosphorylation or dinitrophenyl-derivatized tryptophan.

11. I did not, however, think of the SISCAPA method of using anti-peptide antibodies specific to tryptic peptide sequences to isolate specific signature peptides for quantitation. In fact, it was not apparent that this approach would work in practice given the limited information available regarding binding of tryptic peptides to antibodies.

12. Moreover, not only did the Geng paper not suggest the SISCAPA approach to me, as shown by my remark to Dr. Anderson in 2005, but I do not believe it would reasonably have suggested the SISCAPA method to a scientist in the field in 2003. The SISCAPA approach uses my idea of quantitating proteins via mass spectrometric measurement of signature peptides relative to an isotopically labeled internal standard but combines this with a separation method that differs significantly from the methods used in Geng, and that is not suggested in Geng.

12. Thus, SISCAPA uses antibodies specific to peptides of a particular species. By contrast, the approach employed in Geng used an affinity agent, a lectin, which is not specific for the species of peptide being selected, but rather for carbohydrate moieties that are linked to amino acid side chains on a host of peptides having different sequences.

13. The result of using a lectin affinity agent (isolation of a *class* of peptides) is quite different than the result obtained with antibodies that target specific peptide sequences (isolation of specific peptides). These different results reflect the different goals of the methods described in Geng publication and the SISCAPA method. The goal of the methods described in Geng was proteomic studies directed to identification of unknown proteins in regulatory flux, whereas the SISCAPA method embodied in the claims set forth in Appendix B allows quantitation of a specific peptide present in a sample.

14. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any document or any registration resulting therefrom.

Fred E. Regnier

Dr. Fred Regnier
Date:June 8, 2009

APPENDIX A

Curriculum Vitae
FRED E. REGNIER
John H. Law Distinguished Professor
Department of Chemistry
Purdue University

Address: Fred E. Regnier
Department of Chemistry and
Bindley Bioscience Center
Purdue University
West Lafayette, IN, 47907-1393

Telephone:
Email:
Birthdate:
Marital Status: Married
Soc. Sec. No.:
Citizenship: U.S.A.

Education:

1960	B.S.	Nebraska State College, Peru, NE Chemistry
1965	Ph.D.	Oklahoma State Univ. Stillwater, OK Chemistry
1965	Post doc.	Oklahoma State Univ. Stillwater, OK
1966-67	Post doc.	Univ. of Chicago, Chicago, IL
1968	Post doc.	Harvard Univ. Cambridge, MA

Sabbaticals:

1970 (Summer)	Harvard University, Cambridge, MA
1972 (Summer)	Woods Hole Oceanographic, Woods Hole, MA
1974 (Summer)	Corning Glass Works, Medfield, MA
1992 (Summer)	Massachusetts Institute of Technology, Cambridge, MA
2007 (Fall)	University of Texas Health Science Center, San Antonio, TX
2007 (Fall)	University of California, San Francisco, San Francisco, CA

Research and Professional Experience:

1961-65	Research Assistant, Oklahoma State University
1965-66	Research Associate, Oklahoma State University
1966-67	Research Associate, University of Chicago
1968	Research Associate, Harvard University
1968-71	Assistant Professor of Biochemistry, Purdue University
1971-76	Associate Professor of Biochemistry, Purdue University
1976-77	Associate Director of the Agricultural Experiment Station, Purdue University
1976-1990	Professor of Biochemistry, Purdue University
1990-2004	Professor of Chemistry, Purdue University
2004-present	Distinguished Professor of Chemistry, Purdue University

Societies:
Phi Lambda Upsilon
Sigma Xi
American Chemical Society
American Society of Biological Chemists

Awards and Honors:

David B. Hime Award for Achievement in Chromatography. Presented by the Chicago Chromatography Discussion Group, 1982.
Stephen Dal Nogare Award for Achievements in Chromatography. Presented by the Delaware Valley Chromatography Discussion Group, 1987.
ACS Award in Chromatography. Presented by the American Chemical Society, 1989.
Martin Gold Medal. Presented by the Chromatographic Society of Great Britian, 1993.
ISCO Award. Presented by Instrument Specialties Corporation, 1995.
Pierce Award in Affinity Chromatography. Presented by the International Society for Affinity Chromatography, 1995.
Eastern Analytical Symposium Award for Achievements in Separation Science, 1996.
Distinguished Lecturer, School of Science at the University of Leiden. Annual Scientific Awards Symposium (1999).
CASSS Scientific Achievement Award, 2000.
Golay Award. Presented by the Dutch Chromatography Society, 2001.
Nauta Distinguished Lecturer. University of Leiden. (2004), Netherlands.
Oustanding Commercialization Award, Presented by Purdue University and the Central Indiana Corporate Partnership, 2006.
Edward Herbert Boomer Distinguished Lecturer. (2006), University of Alberta, Edmonton, Canada.

Editoral Boards:

Analytical Biochemistry (1982-1990)
Analytical Chemistry. (1989-1994), (2003 -2007)
Journal of Pharmaceutical and Biomedical Analysis (1989-1996)
Analytical Methods and Instrumentation (1992-1999)
J. Chromatography (1986-1999)
Liquid Chromatography Magazine (1983-Present)
Chimica Oggi/Chemistry Today (1995-1999)
Journal of High Resolution Chromatography (1997-2007)
International Journal of Bio-Chromatography (1995-2007)
Journal of Separation Science (2000-2007)
Pharmagenomics (2002-2007)
J. Proteome Res. (2002-2007)
Bioanalytical Reviews (2008-present)

Journal Articles:

1. **Simultaneous Quantification of Metabolites Involved in Central Carbon and Energy Metabolism Using Reversed-Phase Liquid Chromatography-Mass Spectrometry and in Vitro 13C Labeling.** Yang, Wen-Chu; Sedlak, Miroslav; Regnier, Fred E.; Mosier, Nathan; Ho, Nancy; Adamec, Jiri. Analytical Chemistry, (2008), 80(24), 9508-9516.
2. **Glycoproteomic profiling of serum peptides in canine lymphoma and transitional cell carcinoma.** Wilson, C. R.; Regnier, F. E.; Knapp, D. W.; Raskin, R. E.; Andrews, D. A.; Hooser, S. B. Veterinary and Comparative Oncology (2008), 6(3), 171-181.
3. **GAPDH Is Conformationally and Functionally Altered in Association with Oxidative Stress in Mouse Models of Amyotrophic Lateral Sclerosis.** Pierce, Anson; Mirzaei, Hamid; Muller, Florian; De Waal, Eric; Taylor, Alexander B.; Leonard, Shanique; Van Remmen, Holly; Regnier, Fred; Richardson, Arlan; Chaudhuri, Asish. Journal of Molecular Biology (2008), 382(5), 1195-1210.
4. **Protein:protein aggregation induced by protein oxidation.** Mirzaei, Hamid; Regnier, Fred. Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences (2008), 873(1), 8-14. .
5. **Stable isotope-coded quaternization for comparative quantification of estrogen metabolites by high-performance liquid chromatography-electrospray ionization mass spectrometry.** Yang, Wen-Chu; Regnier, Fred E.; Sliva, Dan; Adamec, Jiri. Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences (2008), 870(2), 233-240.

6. **Use of Glycan Targeting Antibodies To Identify Cancer-Associated Glycoproteins in Plasma of Breast Cancer Patients.** Cho, Wonryeon; Jung, Kwanyoung; Regnier, Fred E.. Analytical Chemistry (2008), 80(14), 5286-5292.
7. **Identification of oxidized proteins in rat plasma using avidin chromatography and tandem mass spectrometry.** Mirzaei, Hamid; Baena, Beatriz; Barbas, Coral; Regnier, Fred. Proteomics (2008), 8(7), 1516-1527.
8. **Two-dimensional correlation optimized warping algorithm for aligning GC x GC-MS data.** Zhang, Dabao; Huang, Xiaodong; Regnier, Fred E.; Zhang, Min. Analytical Chemistry (2008), 80(8), 2664-2671. **Comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry peak sorting algorithm.** Oh, Cheolhwan; Huang, Xiaodong; Regnier, Fred E.; Buck, Charles; Zhang, Xiang. Journal of Chromatography, A (2008), 1179(2), 205-215.
9. **Toward chromatographic analysis of interacting protein networks.** Liu, Xiuping; Yang, Wen-chu; Gao, Qiang; Regnier, Fred. Journal of Chromatography, A (2008), 1178(1-2), 24-32.
10. **Differential Metabolomics Using Stable Isotope Labeling and Two-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometry.** Huang, Xiaodong; Regnier, Fred E.. Analytical Chemistry (2008), 80(1), 107-114.
11. **Comparative metabolite profiling of carboxylic acids in rat urine by CE-ESI MS/MS through positively pre-charged and (2)H-coded derivatization.** Yang Wen-Chu; Regnier Fred E; Adamec Jiri Electrophoresis (2008), 29(22), 4549-60.
12. **Enhancement of the LC/MS Analysis of Fatty Acids through Derivatization and Stable Isotope Coding.** Yang, Wen-Chu; Adamec, Jiri; Regnier, Fred E.. Analytical Chemistry (2007), 79(14), 5150-5157.
13. **Destabilization of DJ-1 by Familial Substitution and Oxidative Modifications: Implications for Parkinson's Disease.** Hulleman, John D.; Mirzaei, Hamid; Guigard, Emmanuel; Taylor, Biochemistry (2007), 46(19), 5776-5789
14. **Identification of yeast oxidized proteins.** Mirzaei, Hamid; Regnier, Fred. Journal of Chromatography, A (2007), 1141(1), 22-31.
15. **Neural network prediction of peptide separation in strong anion exchange chromatography.** Oh, Cheolhwan; Zak, Stanislaw H.; Mirzaei, Hamid; Buck, Charles; Regnier, Fred E.; Zhang, Xiang. Bioinformatics (2007), 23(1), 114-118.
16. **A method for the identification of glycoproteins from human serum by a combination of lectin affinity chromatography along with anion exchange and Cu-IMAC selection of tryptic peptides.** Qiu, Ruiqing; Zhang, Xiang; Regnier, Fred E.. Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences (2007), 845(1), 143-150.
17. **Differential phase-contrast BioCD biosensor**. Zhao Ming; Cho Wonryeon; Regnier Fred; Nolte David Applied optics (2007), 46(24), 6196-209.
18. **Adaptive interferometry of protein on a BioCD**. Peng Leilei; Varma Manoj M; Cho Wonryeon; Regnier Fred E; Nolte David D Applied optics (2007), 46(22), 5384-95. .
19. **High-speed interferometric detection of label-free immunoassays on the biological compact disc.** Zhao, Ming; Nolte, David; Cho, Wonryeon; Regnier, Fred; Varma, Manoj; Lawrence, Greg; Pasqua, John. Clinical Chemistry (2006), 52(11), 2135-2140.
20. **Identification and quantification of protein carbonylation using light and heavy isotope labeled Girard's P reagent.** Mirzaei, Hamid; Regnier, Fred. Journal of Chromatography, A (2006), 1134(1-2), 122-133.
21. **Protein-RNA Cross-Linking in the Ribosomes of Yeast under Oxidative Stress.** Mirzaei, Hamid; Regnier, Fred. Journal of Proteome Research (2006), 5(12), 3249-3259.
22. **Primary amine coding as a path to comparative proteomics.** Regnier, Fred E.; Julka, Samir. Proteomics (2006), 6(14), 3968-3979.
23. **Creation of Allotypic Active Sites during Oxidative Stress.** Mirzaei, Hamid; Regnier, Fred. Journal of Proteome Research (2006), 5(9), 2159-2168.
24. **Enhancement of Amino Acid Detection and Quantification by Electrospray Ionization Mass Spectrometry.** Yang, Wen-Chu; Mirzaei, Hamid; Liu, Xiuping; Regnier, Fred E. Analytical Chemistry (2006), 78(13), 4702-4708.

25. **Enhancing Electrospray Ionization Efficiency of Peptides by Derivatization.** Mirzaei, Hamid; Regnier, Fred. Analytical Chemistry (2006), 78(12), 4175-4183.
26. **Phase-contrast BioCD: High-speed immunoassays at sub-picogram detection levels.** Zhao, Ming; Peng, Leilei; Cho, W.; Regnier, F.; Nolte, D. D. Proceedings of SPIE-The International Society for Optical Engineering (2006), 6095 (Nanobiophotonics and Biomedical Applications III),
27. **Identification of Rotenone-Induced Modifications in α -Synuclein Using Affinity Pull-Down and Tandem Mass Spectrometry.** Mirzaei, Hamid; Schieler, Jeremy L.; Rochet, Jean-Christophe; Regnier, Fred. Analytical Chemistry (2006), 78(7), 2422-2431.
28. **Enrichment of Carbonylated Peptides Using Girard P Reagent and Strong Cation Exchange Chromatography.** Mirzaei, Hamid; Regnier, Fred. Analytical Chemistry (2006), 78(3), 770-778.
29. **Effect of oxidative stress on alpha-synuclein aggregation in Parkinson's disease**. Schieler J L; Liu F; Mirzaei H; Bernas T S; Robinson J P; Regnier F E; Rochet J-C Nanomedicine (2006), 2(4), 317.
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32. **Soft lithography based micron-scale electrophoretic patterning of purple membrane.** Crittenden, S.; Reifenberger, R.; Hillebrecht, J.; Birge, R.; Inerowicz, D.; Regnier, F. Journal of Micromechanics and Microengineering. (2005), 15(8), 1494-1497.
33. **An Automated Method for the Analysis of Stable Isotope Labeling Data in Proteomics.** Zhang, Xiang; Hines, Wade; Adamec, Jiri; Asara, John M.; Naylor, Stephen; Regnier, Fred E.. Journal of the American Society for Mass Spectrometry. (2005), 16(7), 1181-1191.
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36. **The adaptive BioCD: Interferometric immunoassay on a spinning disk.** Peng, Leilei; Varma, Manoj M.; Regnier, Fred E.; Nolte, David D. Proceedings of SPIE-The International Society for Optical Engineering (2005), 5692, 224-232.
37. **Use of Multidimensional Lectin Affinity Chromatography in Differential Glycoproteomics.** Qiu, Ruiqing; Regnier, Fred E. Analytical Chemistry (2005), 77(9), 2802-2809
38. **Affinity chromatographic selection of carbonylated proteins followed by identification of oxidation sites using tandem mass spectrometry.** Mirzaei, Hamid; Regnier, Fred. Analytical Chemistry. (2005), 77, 2386-2392.
39. **Use of multidimensional lectin affinity chromatography in differential glycoproteomics.** Qiu, Ruiqing; Regnier, Fred E. Analytical Chemistry. (2005), 77(9), 2802-2809.
40. **Quantification of phosphoproteins with global internal standard technology**. Riggs, Larry; Seeley, Erin H.; Regnier, Fred E. Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences (2005), 817(1), 89-96.
41. **Reduction of non-specific binding in Ga(III) immobilized metal affinity chromatography for phosphopeptides by using endoproteinase glu-C as the digestive enzyme**. Seeley, Erin H.; Riggs, Larry D.; Regnier, Fred E. Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences (2005), 817(1), 81-88.
42. **Structure specific chromatographic selection in targeted proteomics**. Mirzaei, Hamid; Regnier, Fred. Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences (2005), 817(1), 23-34.
43. **The BioCD: A high-sensitivity spinning-disk interferometer for antigen detection**. Varma, M. M.; Nolte, D. D.; Inerowicz, H. D.; Regnier, F. E . Trends in Optics and Photonics (2004), 96/B.
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David D. Special Publication - Royal Society of Chemistry (2004), 297(Micro Total Analysis Systems 2004, Volume 2), 195-200.

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46. **Analytical techniques**. Zenobi, Renato; Regnier, Fred. Current Opinion in Chemical Biology (2004), 8(5), 517-518.
47. **Spinning-disk laser interferometers for immuno-assays and proteomics: the BioCD.** Nolte, David D.; Varma, Manoj M.; Peng, Leilei; Inerowicz, Halina D.; Regnier, Fred E . Proceedings of SPIE-The International Society for Optical Engineering (2004), 5328 41-48.
48. **Real-time spinning-disk interferometric immunoassays**. Varma, Manoj M.; Inerowicz, Halina D.; Regnier, Fred E.; Nolte, David D. Proceedings of SPIE-The International Society for Optical Engineering (2004), 5328 62-68.
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50. **Benzoyl Derivatization as a Method To Improve Retention of Hydrophilic Peptides in Tryptic Peptide Mapping**. Julka, Samir; Regnier, Fred E . Analytical Chemistry (2004), 76(19), 5799-5806.
51. **Enrichment of cysteine-containing peptides from tryptic digests using a quaternary amine tag**. Ren, Diya; Julka, Samir; Inerowicz, Halina D.; Regnier, Fred E . Analytical Chemistry (2004), 76(15), 4522-4530.
52. **Proteomic Analysis of Arabidopsis Glutathione S-transferases from Benoxacor- and Copper-treated Seedlings**. Smith, Aaron P.; De Ridder, Ben P.; Guo, Woei-Jiun; Seeley, Erin H.; Regnier, Fred E.; Goldsbrough, Peter B. Journal of Biological Chemistry (2004), 279(25), 26098-26104.
53. **Proteomic analysis of carbonylated proteins in two-dimensional gel electrophoresis using avidin-fluorescein affinity staining**. Yoo, Byoung-Sam; Regnier, Fred E. Electrophoresis (2004), 25(9), 1334-1341.
54. **New Approach for Analysis of the Phosphotyrosine Proteome and Its Application to the Chicken B Cell Line, DT40**. Zolodz, Melissa D.; Wood, Karl V.; Regnier, Fred E.; Geahlen, Robert L. Journal of Proteome Research (2004), 3(4), 743-750.
55. **Integrative Biological Analysis of the APOE*3-Leiden Transgenic Mouse**. Clish, Clary B.; Davidov, Eugene; Oresic, Matej; Plasterer, Thomas N.; Lavine, Gary; Londo, Tom; Meys, Michael; Snell, Philip; Stochaj, Wayne; Adourian, Aram; Zhang, Xiang; Morel, Nicole; Neumann, Eric; Verheij, Elwin; Vogels, Jack T. W. E.; Havekes, Louis M.; Afeyan, Noubar; Regnier, Fred; Van Der Greef, Jan; Naylor, Stephen. OMICS (2004), 8(1), 3-13.
56. **High-speed label-free detection by spinning-disk micro-interferometry**. Varma, Manoj M.; Inerowicz, Halina D.; Regnier, Fred E.; Nolte, David D. Biosensors & Bioelectronics (2004), 19(11), 1371-1376.
57. **Effect of microenvironment pH of aluminum hydroxide adjuvant on the chemical stability of adsorbed antigen**. Wittayanukulluk, Arunee; Jiang, Dongping; Regnier, Fred E.; Hem, Stanley L. Vaccine (2004), 22(9-10), 1172-1176.
58. **Contributions of commercial sorbents to the selectivity in immobilized metal affinity chromatography with Cu(II).** Ren, Diya; Penner, Natalia A.; Slentz, Benjamin E.; Inerowicz, Halina D.; Rybalko, Marina; Regnier, Fred E . Journal of Chromatography, A (2004), 1031(1-2), 87-92.
59. **Potential of silica monolithic columns in peptide separations**. Xiong, Li; Zhang, Roujian; Regnier, Fred E . Journal of Chromatography, A (2004), 1030(1-2), 187-194.
60. **Quantification in proteomics through stable isotope coding**. Julka, Samir; Regnier, Fred. Journal of Proteome Research (2004), 3(3), 350-363.
61. **Adaptive spinning-disk interferometry for biomolecule detection.** Peng, Leilei; Varma, Manoj M.; Nolte, David D.; Inerowicz, H. Dorota; Regnier, Fred E. Trends in Optics and Photonics, (2003), 87, 722-728.
62. **Histidine Histidine - rich peptide selection and quantification in targeted proteomics.**

Ren, Diya; Penner, Natalia A.; Slentz, Benjamin E.; Regnier, Fred E.. Department of Chemistry, Purdue University, West Lafayette, IN, USA. Journal of Proteome Research (2004), 3(1), 37-45.

63. **Recognizing single amino acid polymorphism in proteins.** Liu, Peiran; Regnier, Fred E. Analytical Chemistry, (2003), 75(19), 4956-4963.
64. **Comparative proteomics of glycoproteins based on lectin selection and isotope coding.** Xiong, Li; Andrews, Dina; Regnier, Fred. Journal of Proteome Research, (2003), 2(6), 618-625.
65. **Dependence of elution curve and adsorption isotherms of insulin on composition of mobile phase of frontal analysis in reversed phase liquid chromatography.** Geng, Xin-Du; Regnier, Fred E. Chinese Journal of Chemistry, (2003), 21(4), 429-435.
66. **An integrated theory of adsorption and partition mechanism and each contribution to solute retention in reversed phase liquid chromatography.** Geng, Xin-Du; Regnier, Fred E. Chinese Journal of Chemistry, (2003), 21(3), 311-319.
67. **Evaluating immobilized metal affinity chromatography for the selection of histidine-containing peptides in comparative proteomics.** Ren, Diya; Penner, Natalia A.; Slentz, Benjamin E.; Mirzaei, Hamid; Regnier, Fred. Journal of Proteome Research, (2003), 2(3), 321-329.
68. **Regio-specific adsorption of cytochrome c on negatively charged surfaces.** Xu, Wensheng; Zhou, Hong; Regnier, Fred E. Analytical Chemistry, (2003), 75(8), 1931-1940.
69. **Stopped-Flow Enzyme Assays on a Chip Using a Microfabricated Mixer.** Burke, Brian J.; Regnier, Fred E, Analytical Chemistry, (2003), 75(8), 1786-1791.
70. **A direct evidence of stoichiometric displacement between insulin and methanol in reversed phase liquid chromatography.** Geng, Xin-Du; Regnier, Fred E. Chinese Journal of Chemistry, (2003), 21(2), 181-188.
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Appendix B

1-43. (Canceled)

44. (Previously Presented) A method of quantifying an amount of at least a first monitor peptide and a second monitor peptide in a biological sample, comprising:
contacting the sample with

(i) a first anti-peptide antibody specific for said first peptide and;

(ii) a known quantity of a labeled version of said first peptide;

contacting the sample with

(i) a second antipeptide antibody specific for said second peptide, wherein said second antibody is different from said first antibody and;

(ii) a known quantity of a labeled version of said second peptide, separating peptides bound by said first and said second antibodies from unbound peptides;

eluting said peptides bound by said first and said second antibodies from said antibodies;

measuring the amount of said first peptide eluted from said first antibody using a mass spectrometer;

measuring the amount of said labeled version of said first peptide eluted from said first antibody using a mass spectrometer;

calculating the amount of the first peptide in the biological sample;

measuring the amount of said second peptide eluted from said second antibody using a mass spectrometer;

measuring the amount of the labeled version of the second peptide eluted from said second antibody using a mass spectrometer; and

calculating the amount of the second peptide in the biological sample, wherein said biological sample is a proteolytic digest of a bodily fluid sample.

45-47. (Canceled)

48. (Previously Presented) The method of claim 44, wherein at least one of said first and said second antibodies is a monoclonal antibody.

49. (Previously Presented) The method of claim 44, wherein at least one of said first and said second antibodies is a polyclonal antibody.

50. (Previously Presented) The method of claim 44, wherein said first and said second antibodies are both polyclonal antibodies.

51. (Previously Presented) The method of claim 44, wherein said first and said second antibodies are both monoclonal antibodies.

52-53. (Canceled)

54. (Previously Presented) The method of claim 44, wherein the labeled version of the first peptide includes at least one site at which a stable isotope is substituted for the corresponding predominant natural isotope in more than 98% of peptide molecules.

55. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to a support.

56. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to a packed column.

57. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to a monolithic porous support.

58. (The method of claim 44, further comprising: attaching the first antibody to a mesh.

59. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to magnetic beads.

60. (Previously Presented) The method of claim 44, wherein the first peptide and the second peptide are selected from among the set of peptides produced by digestion of the target protein to provide high signal to noise in the mass spectrometer.

61. (Previously Presented) A method for quantifying the amount of a peptide, comprising:
contacting the sample with
(i) an anti-peptide antibody specific for said peptide;
(ii) a known quantity of a labeled version of the peptide,
separating peptides bound by said antibody from unbound peptides
eluting said peptide bound by said antibody from said antibody;
measuring the amount of the peptide eluted from said
antibody using a mass spectrometer: and
calculating the amount of the peptide in the biological sample;
wherein said biological sample is a proteolytic digest of a bodily fluid.

62-63. (Canceled)

64. (Previously Presented) The method of claim 61, further comprising: preparing the labeled version of the peptide.

65. (Previously Presented) The method of claim 61, wherein the labeled version of the peptide includes at least one site at which a stable isotope is substituted for the predominant natural isotope in more than 98% of peptide molecules.

66-70. (Canceled)

71. (Currently Amended) The method of claim 44, further comprising:
preparing the labeled version of the monitor peptide.

72. (Currently Amended) The method of claim 71, wherein the labeled version of the monitor peptide includes a stable isotope.

73. (Canceled).

74. (Previously Presented) method of claim 44, wherein said first anti-peptide antibody is created using said first peptide or a nonmaterially modified version of the first monitor peptide.

75. (Previously Presented)) The method of claim 44, further comprising: creating the first antibody using the first peptide or a non-materially modified version of the first peptide.

76. (Canceled).

77. (Previously Presented) The method of claim 61, further comprising: creating the anti-peptide antibody using the peptide or a non-materially modified version of the peptide.

78. (Currently Amended) The method of claim 44, wherein the said bound peptides are subjected to a chromatography step after elution from said antibodies and before introduction into said mass spectrometer.

79-80. (Canceled)

81. (Currently Amended) The method of claim 61, wherein said bound peptides are subjected to a chromatography step after elution from said antibody and before introduction into said mass spectrometer.

82. (Previously Presented) The method of claim 61, wherein the anti-peptide antibody is a polyclonal antibody.

83. (Previously Presented) The method of claim 61, wherein the anti-peptide antibody is a monoclonal antibody.

84. (Previously Presented) The method of claim 44 wherein said first and second peptides are proteolytically cleaved from first and second sample proteins, respectively, and wherein the amounts of said first and second proteins in said body fluid sample are calculated from the amounts of said first and said second peptides in the sample.

85. (Previously Presented) The method of claim 61 wherein said first and second peptides are proteolytically cleaved from first and second sample proteins, respectively, and wherein the amounts of said first and second proteins in said body fluid sample are calculated from the amounts of said first and said second peptides in the sample.

86. (Previously Presented) The method of claim 61, wherein the polyclonal antibody is created using the monitor peptide or a non-materially modified version of the monitor peptide.